October 4, 1972

Re: Case No 135 "Effect of Nicotine on the Rat Fetus" 1/1/73 - 12/31/75

Supporting Statement for Application

Justification of budget:

Personnel:

H.D. Mosier, investigator: A $5,000 salary is requested to relieve Memorial HOspital of Long Beach of this portion of its salary obligation. The amount is approximately 13% of the combined current salary made up by the University base salary and the Memorial Hospital supplement. It does not entirely balance the 40% estimated for the project, but will relieve the investigator of some administrative and teaching obligations and enhance attainment of the project goals.

Regina A. Jansons: Miss Jansons has a B.S. degree and ten year's experience. Seven of these are in the investigator's laboratory in directly related work. The slalry is in line with her current salary plus expected merit and cost of living increases anticipated in accord with Memorial Hospital policy. Miss Jansons has considerable experience in management of the animal colony, breeding techniques, measurement in vivo, dissections, homogenizations, extractions, thin layer chromatography, radioisotope determination by scanning, and liquid scintillation techniques, gas chromotography, and miscellaneous biochemical techniques.

Personnel budget provides fringe 6.74% which indludes FICA and state of California Workman's Compensation. A 5% increase is calculated for each year of the project to cover anticipated merit or cost of living increases.

Supplies:

Animal care is the largest item but is somewhat less than actual cost. The balance is absorbed by the budget of the Department of Medical Education at Memorial Hospital.

Chemicals and radiochemicals are the next largest item. Radiochemicals are included to provide for the purchase of labelled nicotine to be used in some studies to determine the proportion of nicotine absorbed from diet when it has been mixed with the food in accord with our experiments involving the intake of nicotine throughout pregnancy.

Other expenses:

Equipment maintenance and service contracts: We have sufficient equipment in the laboratory to carry out the present study; however; the cost of maintenance and service contracts for some of the large pieces of equipment

is a significant item. These pieces of equipment are the Aminco-Bowman spectrophotofluorometer, Hewlett-Packard Model 9100 B programmable calculator, Beckman DU-2 spectrophotometer, and a Packard Instrument Company Model 3320 Liquid Scintillation Counter.

Relevance of this work to research on Tobacco and Health:

Transplacental passage of nicotine has been demonstrated by a number of investigators; early work has been reviewed by Larson et al. (1). Investigations after injection of (-)-nicotine-N-methyl-^{14}C into pregnant mice have shown the presence of labelled nicotine, cotinine, and other metabolites in fetal tissues (2,3). Evidence has been put forward suggesting that the decidua basalis bars free transplacental diffusion of nicotine (3). In vitro incublation of slices showed no evidence of formation of metabolites by placenta or fetal lung and only traces of cotinine in fetal liver suggesting that fetal tissues had relatively less ability than adult tissues to form metabolites of nicotine (3).

It has been recognized in studies with ^{14}C-nicotine, labelled in the N-methyl group, that demethylated derivatives cannot be detected (2). The advantage of a general label was shown in the experiments of Bowman et al. who were able to show a significant fraction of ^{3}H-demethylcotinine in the urine of mice injected with ^{3}H-cotinine (4).

The acute administration of nicotine in pregnant animals has been shown to produce capillary damage in dog placentas (5), teratogenic effects on the skeletal system of the offspring and decrease of litter size in mice (6), vertebral anomalies in chicks (7), and postponement of the appearance of the first litter and lighter weight offspring in rats (8,9). The administration of nicotine to nursing rat mothers is associated with a poor neonatal weight gain and an increase in neonatal mortality (9,10). Geller (11) gave pregnant rats nicotine doses less than 15 percent of those used by Hishimura and Nakai in mice (6) and produced no fetal abnormalities. This difference may be explained on the basis of dosage and species differences.

Both acute and chronic exposure to nicotine have long been known to produce changes in lipid metabolism. Acute administration of nicotine results in a rise in serum free fatty acids (FFA) in dogs and humans (12). Smokers, when compared with non-smokers, have been shown to have increased serum lipoproteins (13) and cholesterol (13-18). However, in some studies there has been no difference observed in cholesterol levels between smoking men in the age range 18-25 (19) and in the age range of 65-86 (20). These differences have not been satisfactorily accounted for.

It is probable that some of the changes in lipid metabolism in the adult are indirectly produced through the nicotine effect on the adrenal medulla causing

a release of epinephrine (21-23). When epinephrine in oil is administered, there is an immediate lipid mobilization followed in 24 to 48 hours by a rise to peak levels of serum lipoproteins, cholesterol and phospholipids (22-24). These effects may be independent actions of the hormone since the rise of lipoproteins will occur when the FFA response is blocked by glucose or insulin (24). Both responses are abolished by hypophysectomy or adrenalectomy, but can be re-established with cortisone (25). Chronic nicotine intake may produce hyperlipemia in the adult through repeated release of epinephrine and the effect of subsequent repeated rises of FFA on the liver or through some independent action causing a rise in lipoproteins.

Adrenergic blocking agents can inhibit the rsie of FFA which result from epinephrine or norepinephrine administration (26). It has recently been shown that the beta adrenargic blocking agents nethalol and a cnetral adrenergic reflex blocking agent, chlorpromazine, are able to block FFA rise after nicotine and inhibit to some extent triglyceride rise. Phenoxybenzamine, an alpha blocker, did not have this action (27). One might infer from this that epinephrine or isopropyl-norepinephrine mediate the release of FFA by nicotine.

A direct nicotine effect in lipid metabolism on the cellular level is implied in recent experiments in dogs in which chronic administration of nicotine resulted in a selective diminution of acetate 1-C^{14} incorporation into cholesterol and reduced cholesterol turnover (28). The significance of these effects in overall lipid metabolism remains to be determined.

Smoking during pregnancy in the human has been rleated to an increased incidence of prematurity and low birth weight infants in a number of studies (29-33). Whether this is a direct effect of tobacco products on fetal growth and the physiology of pregnancy or indirect through such factors as maternal diet cannot be determined with the present data. Our own experiments suggest that maternal dietary disturbance may be one of the elements in the production of low birth weight by tobacco smoking. This conclusion is compatible with recent reports of low birth weight and immaturity of pups of rats given large doses of nicotine during pregnancy. The maternal food intake and weight gain were reduced concomitantly (34).

A number of workers have related smoking or nicotine intake to cardiovascular disorders. A recent review dealt with smoking as a factor in atherosclerosis (35). Chronic administration of nioctine will produce a variety of aortic lesions in adult rabbits. In a recent study, it was shown that there are intimal deposits of mucopolysaccharides and medial lesions with localized necrosis, fibrosis, and calcification (36). When nicotine is administered either intravenously or directly, the microcirculation responds by initial vasoconstriction, then vasodilation. Arteriolar walls thicken (37). Rabbits who have remained without disease after receiving definite doses of vitamin D and dietary cholesterol for many months respond to the addition of nicotine with fatal calcific arterial lesions especially conspicuous in peripheral arteries (38). There medial calcific degeneration of arteries is accompanied

by fibrocellular proliferation of the media. The mesenchymal reaction attracts xanthomatous accumulations which appear in the thickening intima at serum cholesterol levels no greater than found commonly in man. The changes also occur in cardiac muscle arteries. A recent study showed that subendothelial fibrosis in rabbit arteries after chronic nicotine dosage is inhibited by administration of a mono amine oxidase inhibitor. The authors propose the hypothesis that the monoamine oxidase inhibitor inhibits the action of epinephrine in the production of the arterial lesions.

Generalized calcific arterial disease has long been noted in human infants (39). The etiology is unknown and the disorder is mentioned here only to indicate that generalized arteriopathy occurs in the human infant. Many observations have been made in this condition which have been summarized in two textbooks of pediatric pathology (40,41) and reviewed recently in tow case reports (42, 43). A detailed study in one of these (43) brought out evidence suggesting that mediocalcinosis in infants is a primary elastic tissue disorder with accumulations of mucoid followed by calcification. The intimal fibrosis is seen as a secondary change.

The pharmacology of nicotine has been extensively studied (1). Recent work has been summarized in the Fourth International Symposium at the Wenner Gren Center, Stockholm, 1964 (44). In this, the work of various investigators of the metabolism of nicotine in tissues was brought together. Nicotine injected into the mouse is rapidly accumulated in brain, adrenal medulla and superior cervical ganglion. The accumulation in the brain disappears in 30-60 minutes (45,46). In the rabbit liver 8 metabolites of nicotine have been demonstrated after in vitro or in vivo exposure (47, 48). Both rat and rabbit excrete a misture of pyridine compounds in the urine. Cotinine is common to both rat and rabbit urine; 8 pyridine compounds have been isolated from rate urine after nicotine (49). In the mouse, nicotine is rapidly metabolized to cotinine in tissues and in one study $C^{14}O_2$ was the only other radioactive compound after administration of C^{14} nicotine. A number of metabolic products were identified by chromatographic methods (50,51).

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